

AMENDMENT

In the Specification:

Please amend the paragraph beginning at page 1, line 9 as follows:

This application is a continuation of U.S. Patent Application Serial No. 09/706,580, filed November 3, 2000 which is related to provisional patent application serial no. 60/164,068, filed November 5, 1999, from which priority is claimed under 35 U.S.C. ' 119(e)(1) and which applications is are incorporated herein by reference in ~~its~~ entirety their entireties

Please enter the accompanying initial paper copy of the "Sequence Listing" into the specification.

Please amend the paragraph beginning on page 30, line 7 as follows:

p4.1c: A synthetic DNA encoding the restriction enzyme sites NotI-MluI-Ecl136II-SstII-SfuI-SmaI-SfuI-ClaI-BglII-SnaBI-BstEII-PmlI-RsrII-NotI and having the sequence
(CGGCCGCACGCGTGAGCTCCGCGGTTCGAATCCCGGGATTCTGAACATCGATA
AAAGATCTACGTAGGTAACCACGTGCGGACCGAGCGGCCGC) (SEQ ID NO:1)
was cloned into the blunted KasI and EarI(partial) sites of pUC119 (the vector fragment is 2757bp in length). A 653bp SpeI(blunted)-SacII(blunted) fragment encoding the CMV immediate early (IE) promoter, and a 488bp, SmaI-DraIII fragment containing the human growth hormone polyadenylation site, were cloned into the Ecl136II and SnaBI sites of the afore-mentioned plasmid, respectively. A chimeric intron composed of the splice donor from the first intron of CMV IE gene and the splice acceptor from the second intron of the human β - globin gene was then installed into the SmaI site of the plasmid in two steps. A DNA fragment encoding the CMV IE gene first intron splice donor was produced by PCR using isolated CMV DNA (strain ad169) as template and the following

primers, GGCCGGGAACGGTGCATT (SEQ ID NO:2), and GGGCAAGGGGGTGGGCCTATA (SEQ ID NO:3). This 87 bp fragment was ligated into the SmaI site of the plasmid intermediate. The resulting plasmid was cleaved with BstXI and SmaI, blunted with T4 DNA polymerase, and a 398bp DraI-EcoRI(blunt) fragment encoding the human β -globin second intron splice acceptor was ligated into the plasmid. The construction of p4.1c was completed by ligation of a polylinker encoding the restriction sites ClaI-EcoRI-SmaI-BamHI-XbaI-SalI-PstI-HinDIII-XhoI-Eco47III-XhoI-BglII between the ClaI and BglII sites of the last intermediate plasmid. The sequence of this synthetic DNA was ATCGATTGAATTCCCCGGGGATCCTCTAGAGTCGACCTGCAGAAGCTTGCTCTCGAGCAGCGCTGCTCGAGAGATCT (SEQ ID NO:4).

Please amend the paragraph beginning on page 31, line 1 as follows:

p4.1c mEPO: p4.1c was digested with SmaI and a 2812bp SmaI(partial)-NcoI(blunted) fragment encoding all of the exons of the mouse erythropoietin gene was inserted. The Kozak sequence around the initiator methionine was changed to the optimally translated sequence, CCACCATG (SEQ ID NO:5), using oligonucleotide directed mutagenesis. The sequence of the mutagenic oligonucleotide was AGCTAGGCGCCACCATGGGGGTGC (SEQ ID NO:6).

Please amend the paragraph beginning on page 31, line 8 as follows:

pV4.1c mEPO: The polylinker and lacZ alpha fragment expression cassette of pUC119 was replaced by a single Sse8387I site by ligation of the following synthetic DNA fragment in the plasmid vector after digestion with AflIII and EheI, GGCGCCCCTGCAGGACATGT (SEQ ID NO:7). The resulting plasmid was cut with Sse8387I and the 4772bp Sse8387I fragment from pW1909adh lacZ that contains the ITR-bounded lacZ expression cassette was ligated to it. The resulting plasmid was called intermediate1. Next, p4.1c mEPO was digested with NotI and the 4582bp fragment encoding the mEPO expression cassette was isolated. One copy of a synthetic DNA

fragment that encodes the D region of the AAV ITR was ligated to each end. The sequence of this synthetic fragment was GCGGCCGCAGGAACCCCTAGTGATGGAGTTGG (SEQ ID NO:8). The product of this reaction was ligated into the 2831bp, plasmid vector encoding MscI fragment of intermediate1(above) to form pV4.1c mEPO.

Please amend the paragraph beginning on page 31, line 21 as follows:

p4.1c hEPO: p4.1c was cleaved with SmaI and the 718bp, PpuMI-NcoI fragment of the human Epo cDNA (blunted) was ligated into this site. The translational initiation sequence was then modified by oligonucleotide -directed mutagenesis using the following mutagenic oligo: ~~c~~atcgattgaattccaccatgggggt
CATCGATTGAATTCCACCATGGGGGT (SEQ ID NO:9). The resulting construct was cleaved with Pml I and the 1765bp, EcoRV-HincII fragment of the LacZ gene was ligated into it.

After page 45, please insert the following:

-- Sequence Listing--

then insert the 3 pages of the sequence listing.